By the present invention, there are provided methods for inducing apoptosis of a selected group of vertebrate cells, e.g., a tumor, *in vivo*, by administering an attenuated non-pathogenic bacterium comprising a recombinant nucleic acid molecule encoding said thiaminase targeted to said selected group of vertebrate cells, thereby the level of thiamin in said cells is reduced sufficiently to induce apoptosis of said cells.

By the present communication, Claims 1, 3, 10-11, 18, and 25-27 have been amended to define Applicants' invention with greater particularity. No new matter is introduced by the subject amendments as described herein as the amendments are fully supported by the specification as filed. Claims 28-31 have been cancelled without prejudice. New Claim 32, which depends from amended Claim 25, has been introduced in order to provide greater particularity and to afford parallel construction to claims 26-27, currently amended. No new matter is introduced by new Claim 32.

A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented in the Listing of Claims commencing on page 2, with an appropriate defined status identifier. Claims 1, 3, 10-11, 16-27, and 32 are pending, with claims 16, 17, and 20-24 withdrawn as being drawn to non-elected matter, and claims 1, 3, 10-11, 18, 19, and 25-27, and 32 under active consideration. Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Provisional Double Patenting Rejection

The provisional rejection of claims 1, 3, 10, 11, and 25-27 under the judically created doctrine of obviousness-type double patenting as being unpatentable over claims 1-7, and 15-20 of copending U.S. App. No. 10/342,119 is respectfully traversed. The present invention, as described for example by claim 1 as amended, provides for the administration of a non-

pathogenic bacterium comprising a recombinant nucleic acid molecule encoding a thiaminase from *N. gruberi*. In contrast, the '119 applications provides for the administration of at least one thiamin-depleting agent selected from the group consisting of a thiamin antimetabolite, a thiamin-cleaving compound, and a gene that encodes a polypeptide that acts as a thiamin-depleting agent. Accordingly, in view of the requirement for a non-pathogenic bacterium component of the present invention, the '119 application does not disclose or suggest all of the claim elements of the present invention. In the alternative, to the extent that the present rejection could be overcome by the timely filing of a Terminal Disclaimer, Applicants defer submission of a Terminal Disclaimer pending resolution of the claim elements.

Rejection under 35 USC § 112, First Paragraph, of claims 1, 3, and 25-31.

The rejection of claims 1, 3, and 25-31 under 35 USC § 112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention, and wherein the rejection is a new matter rejection for allegedly containing subject matter not described in the specification as filed, i.e., requirement for non-pathogenicity and methods of use, is respectfully traversed.

Claim 1, as amended, provides for a method of the discovery of the Applicants: "localized apoptosis induced by depletion of thiamin" (LAIDT.) Specifically, the instant application provides for depletion of thiamin by the action of a thiaminase expressed in non-pathogenic bacteria designed to target the tumor sites (specification page 19, lines 22-24.) Indeed, the term "non-pathogenic" and surrogate terms thereof, e.g, avirulent, attenuated, etc., are found throughout the specification, all in the context of bacterial delivery of thiaminase and induction of apoptosis without unwanted sepsis (specification page 36, line 28): e.g., "attenuated (non-pathogenic) hyperinvasive, polyauxotrophic mutants of *S. typhimuriam,*" page 34, line 28; attenuated mutants of *Salmonella*, page 35, line 8; "attenuated, hyperinvasive bacterial strains," page 35, line 12; and "avirulent *C. sporogenes,*" page 36, line 21. Accordingly, the term "non-

pathogenic" is used consistently throughout the specification to refer to bacteria that have been rendered, or alternatively, are inherently not pathogenic.

In the context of LAIDT, Claim 1, as amended, provides a method of inducing apoptosis of a selected group of vertebrate cells *in vivo*, comprising administering to a vertebrate comprising said cells a non-pathogenic bacterium selected from the group consisting of *C. sporogenes*, *C. beijerinckii*, and *S. typhimurium* comprising a recombinant nucleic acid molecule encoding thiaminase I from *N. gruberi* targeted to said selected group of vertebrate cells, thereby reducing the level of thiamin in said cells sufficiently to induce apoptosis of said cells.

Support for the requirement of non-pathogenicity of *C. sporogenes* and *C. beijerinckii* recited in Claim 1 is found in the specification for avirulent *C. sporogenes* (specification page 36, line 21) and avirulent *C. beijerinckii* (specification at, e.g., page 36, lines 12-15.) Also described in the context of Clostridia are techniques for the genetic engineering which have long been known in the art, e.g., Rood et al., 1997 (specification, page 36, lines 14-15.) Furthermore, support for the requirement for non-pathogenicity of *Salmonella* is provided, e.g., in the specification at page 35, lines 12-14. Applicants submit that mutation of *Salmonella*, and in particular *S. typhimurium*, to provide attenuated non-pathogenic species (specification page 34, lines 27-28) for use in methods of the invention is long known in the art, e.g., Pawelek et al., 1997, specification page 34, lines 27.

In the context of the thiaminase element of the invention, the allegation that the claimed embodiments lack sufficient written description support for a non-pathogenic bacterium comprising a recombinant nucleic acid encoding a thiaminase and methods of use thereof for inducing apoptosis in vertebrate cells (Office Action, page 7, lines 4-7) is respectfully traversed. First, the specification teaches that thiaminase can induce apoptosis in vertebrate cells; see e.g., specification at page 33, lines 30-32, which states "[t]he many cell lines in which the *Naegleria* agent (thiaminase I) was shown to induce apoptosis include two cell lines derived from human

prostate cancers." Second, as described by the present communication, the specification provides description of non-pathogenic bacteria carrying thiaminase. Indeed, Example 6 (specification pages 33-36) provides an example of thiaminase incorporated into non-pathogenic bacteria used for prostate cancer therapy. Third, examples of the engineering of a thiaminase into attenuated Salmonella and Closteria are provided in the specification at page 35, lines 12-18, and page 36, lines 28-31, respectively.

One of skill in the art would recognize that a bacterium expressing thiaminase could therefore induce apoptosis in vertebrate cells. Thus, the specification provides adequate support for claimed methods in accordance with 35 U.S.C. § 112.

Accordingly, Applicants submit that the allegation that

> ...these amendments introduce new matter into the disclosure, and that the claimed invention as a whole is <u>not</u> adequately described if the claims require essential, or critical elements which are not adequately described in the specification **and which are not conventional in the art as the Applicants' effective filing date.**

(Office Action, page 6, lines 13-17, emphasis added) is defective because the methodology for achieving non-pathogenicity of, and the incorporation of a thiaminase into, *C. sporogenes*, *C. beijerinckii*, and *S. typhimurium* were well known in the art prior to the filing date of the instant application. Thus, possession of the invention has been shown by description with sufficient, relevant, identifying characteristics such that a person skilled in the art would recognize that the inventors had possession of the claimed invention. Accordingly, reconsideration and withdrawl of the present rejection are respectfully requested.

Rejection under 35 USC § 112, First Paragraph, of claims 1, 3, 11, 18, 19, and 25-31.

The rejection of claims 1, 3, 11, 18, 19, and 25-31 under 35 USC § 112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention, is respectfully traversed.

Specifically, the rejection is directed to claims directed to a thiaminase, derivatives thereof, and sequences that are variably identical to a thiaminase.

In view of the admission of the Examiner that thiaminase I from *N. gruberi*, as encoded by SEQ ID NO: 3, has been adequately described (Office Action, page 8, lines 8-9), and in an effort to reduce issues and expedite prosecution, claims 1, 3, 11, 18 and 25 have been amended to provide that the thiaminase of the invention is thiaminase I from *N. gruberi*. The amendments to claim 18 additionally finds support in the specification (page 11, lines 21-25) for the recitation of 90% homology over 200 nucleotides. Additionally, claim 18 has been amended to recite *N. gruberi* rather than the full genus-species name in parallel with the use of such art-recognized abbreviation throughout the claims. Accordingly, reconsideration and withdrawl of the present rejection are respectfully requested.

Rejection under 35 USC § 112, First Paragraph, of claims 1, 3, 11, and 26-31.

The rejection of claims 1, 3, 11, and 26-31 under 35 USC § 112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention, is respectfully traversed. Specifically, the Examiner asserts that the claimed invention allegedly encompasses gene therapy (Office Action, page 13, line 5) and that the specification fails to provide guidance or working examples with regard to the unpredictabilities associated with gene therapy.

Applicants reiterate the assertion that the pending claims in question neither recite the replacement of a defective gene nor the transfection of cells with a gene in order to achieve the desired result of inducing apoptosis in those cells, wherein replacement of a defective gene and/or transfection of cells with a gene in order to achieve apoptosis of those cells are hallmarks of gene therapy. On the contrary, the present invention can properly be viewed as a method of reducing thiamin by a mechanism external to the cells of the subject vertebrate. Thus, in analogy

to a claim contemplating a pharmaceutical, the present invention provides a method of inducing apoptosis *in vivo* by reducing the level of thiamin. More specifically, the non-pathogenic bacterium expressing thiaminase of the invention fulfills exactly the same role in the induction of apoptosis as would a small molecule pharmaceutical which sufficiently reduced the level of thiamin in the cell.

In this context, the specification provides multiple examples of target cells, e.g., upstream of a capillary bed feeding a tumor (specification page 32, line 13), specific cavities in the body such as the bladder or colon (specification page 32, lines 27-28), the lung (specification page 32, line 29), and solid tumor (specification page 32, line 21.) Furthermore, the specification provides multiple methods of contacting including instillation (specification page 34, line 23), pulmonary absorption (specification page 32, line 29), and injection (specification page 35, line 14.) Finally, the specification provides guidance regarding targeting of thiaminase by e.g., localized administration (specification page 32, line 13) in the capillaries and lung, and by administration to the hypoxic environment of solid tumors. Accordingly, reconsideration and withdrawl of the present rejection are respectfully requested.

In the event that any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

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The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

Date November 7, 2005

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